

AIR MAIL

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Dated.....196

28th September, 1970.

फि०/७०-७१/१०६

Dear Maxine,

Many many thanks for your letter dated September 14. I have informed Koteswar about the staining procedure. Poly A solutions, though slightly viscous, are working alright.

We have already commercial samples of poly I, poly C and alternate poly AU. So we are ready to search for RNase III type of activity. In case you have already started please let us have your advice. I am also keeping track of Schlessinger's reports. Unfortunately, however, our ultracentrifuge which arrived about a month ago is not yet put into operation. As soon as it is working we hope to look for similar system in S.typhimurium. Yes we know that S.typhimurium RNase II is extremely thermolabile. So we can easily get rid of it.

Perhaps you have forgotten that we use phage P22-infected spheroplasts as our starting material for our RNase II preparation (B.B.R.A., 212, 102, 1970) for two reasons. On conversion of cells to spheroplasts there is loss of considerable amount of RNase I, as expected. Further, on infection of these spheroplasts with phage there is still some more lowering. Even the intact cells on phage infection loose some (not much, we did not make quantitative measurements) RNase I. We feel that the enzyme is released due to some type of physical shock as a result of phage infection. There is similar report in literature. In case you are further interested I shall send you the reference.

The enzyme RNase I that gives only 2 bands is not 100 fold purified, it is about 500-1000 fold purified. You have missed the point in the report that the enzyme purified through our standard procedure (J.Bio.Chem., 243, 1133, 1969) was further purified through phosphocellulose column and this column eluate gave only two protein bands. Unfortunately RNase assay on gels is not working in our hands. Have you any experience with it? Please let us know.

Our progress with RNase is slow. With a new colleague (Aloke Dutta) we tried to repeat our earlier results of having multiple peaks of RNase I from DEAE cellulose column. We get 2 to 3 distinct peaks all behaving like RNase I activity but it occurred to us that they may represent free RNase and RNase bound with the ribosomes. Actually DEAE cellulose column has been used for purification of the ribosomes. Since we know that  $Mg^{++}$  is required for the latency of the enzyme we prepared extracts in presence and absence of  $Mg^{++}$ . Elution pattern varies but multiple peaks are there in both the cases. It is unfortunate that the ultracentrifuge is not working. We could have then easily solved the problem. Anyway we then incubated the extracts in presence of EDTA to destroy the ribosomes (we

have indirect evidence that RNase I destroys the ribosomes under these conditions) but still multiple peaks of RNase I are obtained. This may be an artifact. So we are planning to concentrate each peak and put it back on the column to find out whether the multiple peaks really represent multiple forms of RNase I. You mentioned about some report on similar line. Will you kindly send the reference?

Another new colleague of ours (Khandekar) is searching for phage P22-specific DNase in phage-infected S. typhimurium extracts. He is using  $P^{32}$ -DNA as substrate. Large amount of endonuclease (though inhibited by tRNA) and another E. coli exonuclease type of activity are creating problems. We have, however, indication of increase of a DNase activity specific for single stranded DNA. The work is however, in very preliminary stage.

It is now high time for me to plan my trip. So please let me have approximate idea about the timing of Gordon Research Conference and Cold Spring Harbor meeting. Please let me know the name and address of the person to whom to write regarding G.R.C.

Maharani is excited to learn about the robe. I am always excited to receive your letter as it contains lot of food for thinking. So boss! please be regular in correspondence.

Maharani joins with me in sending our best regards to Dan and love to the children.

Sincerely yours,

*Debi'*  
( D. P. Burma )